INTERACTION STUDIES WITH DNA

II. THE BINDING OF "DIMERIC" AND "POLYMERIC" ROSANILINE BY SODIUM THYMONUCLEATE AND THE METACHROMATIC EFFECT

by

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The changes in absorption spectrum of metachromatic basic dyestuffs in aqueous solution and in presence of negatively charged colloids, and their dependence on the ratio of concentration of dyestuff to that of colloid, have been well defined from the qualitative point of view[1], particularly in the case of the interaction between toluidine blue and nucleic acids[2,3,4].

Rosaniline hydrochloride is a basic dye which shows no deviation from Beer's Law[5] in aqueous solution at least up to a concentration of about 10^{-4} M, whereas in presence of *ca.* one equivalent of sodium thymonucleate a strong metachromasy is exhibited[6,7]. This absence of dimerization or polymerisation in aqueous solution simplifies the problem of a more complete analysis of the changes in spectrum associated with metachromasy, and the purpose of the present communication is to suggest such analysis and to report experimental evidence in support of it.

EXPERIMENTAL

Sodium deoxyribonucleate (DNA) was a preparation from calf thymus by the method of BUTLER CONWAY AND JAMES[8] (P = 7.25%, H_2O = 19.9%). Rosaniline hydrochloride (E. Gurr & Co.) was twice recrystallized from water and dried.

Spectrophotometric measurements were made using the Uvispek instrument, with concentrations of rosaniline 1.1, 2.2 and $4.4 \cdot 10^{-5}$ M in ½ and 1 cm cell thickness.

Stock solutions of DNA were 0.2% in $5 \cdot 10^{-3}$ NaCl, and mixtures were made by adding the appropriate volumes of $2.2 \cdot 10^{-4}$ M rosaniline to the diluted DNA solutions.

Results reported were, unless otherwise stated, made with unthermostatted solutions (room temperature 23° C); the effect of raising the temperature of the solutions to 45° C, using a water-heated cell-housing, was not great although always in the direction of decreasing metachromasy *e.g.* with $2.2 \cdot 10^{-5}$ M rosaniline and $2.2 \cdot 10^{-5}$ M DNA, ε at 555 mμ was raised from $35.5 \cdot 10^3$ at 23° C to $38 \cdot 10^3$ at 45° C.

RESULTS

Fig. 1 shows typical absorption spectra due to rosaniline and their dependence on DNA concentration. Curve 1 is that due to rosaniline hydrochloride in water, (concentration denoted by R), with R = 1.1, 2.2 or $4.4 \cdot 10^{-5}$ M. The effect of large

References p. 332.

excess of DNA ($2.42 \cdot 10^{-3}$ M) is shown by curve 2, while curves 3 and 4 show the metachromatic spectra induced by a concentration of DNA approximately equal to that of R (for curve 3, R = $2.2 \cdot 10^{-5}$ M, DNA = $2.42 \cdot 10^{-5}$ M; for curve 4, R = $4.4 \cdot 10^{-5}$ M, DNA = $4.84 \cdot 10^{-5}$ M). Curves 5 and 6 are values of the extinction coefficients for bound "dimeric" and "polymeric" rosaniline derived in the manner to be discussed below.

Figs. 2 and 3 show the variations in ε for rosaniline ($2.2 \cdot 10^{-5}$ M) due to various concentrations of DNA; (abscissae denote the molar fraction R/(R + DNA)).

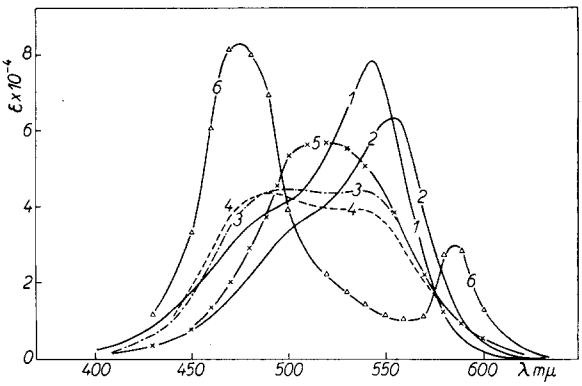

Fig. 1. Absorption spectra of rosaniline hydrochloride: (1) in water, (2) monomeric rosaniline bound to DNA (3) and (4) metachromatic spectra, (5) bound "dimer", (6) bound "polymer".

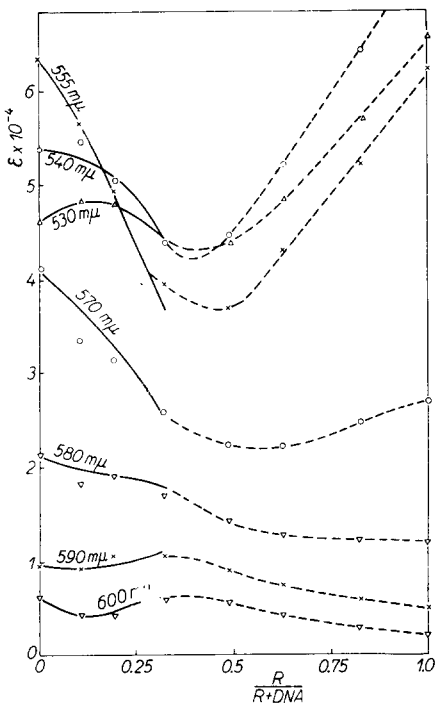

Fig. 2

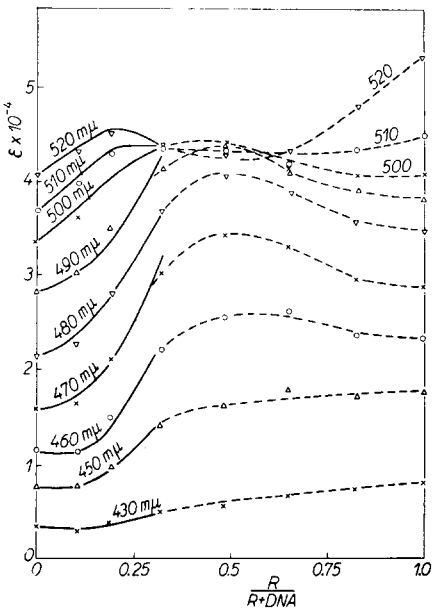

Fig. 3

Figs. 2 and 3. Variation of extinction coefficient due to rosaniline with the ratio of concentrations (rosaniline/rosaniline + DNA).

DISCUSSION

If the distance between the relevant binding sites (positions of negative charge) of a polymer is small (of the order of 3.5 A), the occupation of neighbouring sites by dyestuff cations, if sterically possible, as for those of planar configuration[5] with their mole-

References p. 332.

cular planes approximately at right angles to the polymer surface, will constitute the binding of "dimeric" or "polymeric" species, according as two or more adjacent sites are occupied. As MICHAELIS[9] has pointed out, the configuration of DNA (as the fibrous Na salt) according to the model proposed by ASTBURY[10] (or as modified by CRICK AND WATSON[11]) is such as to present to the solution a surface of high charge density, with an array of primary phosphate groups separated by distances along the polymer chain of *ca.* 3.5 A. MICHAELIS AND GRANICK[12] found that sodium nucleate, in contrast to most negative colloids, gave no metachromasy, or at most a weak effect, when the concentrations of nucleate and dyestuff were equal. However, this effect was due in the main to the lowering of the binding constant for the dyestuff by the presence of a large excess of Na^+ ions[7], as acetate buffer. In absence of added Na^+, a strong metachromasy can be obtained with the usual dyestuffs, but this is displaced by adding metallic cations, as BANK AND BUNGENBERG DE JONG showed[2], divalent cations being some thirty times as effective as monovalent. These effects have been confirmed[3,4].

The origin of the metachromatic effect induced by DNA, (and similar considerations would be expected to apply to soap micelles, where metachromasy is exhibited when the concentration of micellar soap is approximately equal to that of the dyestuff, *i.e.* with small concentration of dye near the critical micelle concentration[13], provided that this is greater than the dye concentration), would appear therefore to be somewhat as follows. For a given value of the degree of binding (β) (where β = the proportion of binding sites, assumed to be equal to the number of phosphate groups, occupied by rosaniline) the bound dye cations will be divisible into three groups, in the proportion $\beta_1:\beta_2:\beta_3$ respectively: (1) monomers, with sites on each side either unoccupied or occupied by Na^+ ions; (2) "dimers", with one neighbouring site occupied by a dye cation; and (3) "polymers", with both neighbouring sites occupied by dye cations. The processes of "dimerization" or "polymerization" here denote juxtaposition of dye cations sufficiently close for Van der Waals' interaction[14], such as to cause changes in the absorption spectrum of the dye.

Making the assumption that the binding sites are equivalent we obtain from simple statistical considerations that:

$$\beta_1 = \beta\,(1-\beta)^2;\quad \beta_2 = 2\beta^2\,(1-\beta);\quad \text{and}\quad \beta_3 = \beta^3.$$

Further, $\beta = \beta_1 + \beta_2 + \beta_3$, may be related to the concentrations of free dye, (R_F), and of Na^+ by the equation[7]:

$$\frac{(1-\beta)}{\beta}\,(R_F) = \frac{1}{k'_R} = \frac{1}{k_R} + \frac{k_{Na}}{k_R}\,(Na^+).$$

The relation between the observed changes in absorption spectrum (observed extinction coefficient = ε) and the ratio of concentrations (R/DNA) is then obtained by introducing the extinction coefficient of free dye (ε°), of bound monomer (ε_1), bound "dimer" (ε_2), and bound "polymer" (ε_3):

$$\varepsilon = \varepsilon^\circ\left(1 - \frac{\beta\cdot DNA}{R}\right) + \varepsilon_1\beta_1\left(\frac{DNA}{R}\right) + \varepsilon_2\beta_2\left(\frac{DNA}{R}\right) + \varepsilon_3\beta_3\left(\frac{DNA}{R}\right).$$

Of these quantities, apart from those known directly, ε_1 can be determined as the value of ε when (R/DNA) approaches zero; and for low ratios (R/DNA), in practice between 0 and 0.4, the binding constant k'_R in absence of added Na^+ (apart from that derived by dilution of the stock DNA) may be assumed large enough for the approximation that all the R is bound, *i.e.* β = (R/DNA), to hold.

References p. 332.

It becomes therefore possible to derive values of ε_2 and ε_3 on these assumptions, and these are shown in Fig. 1 as curves 5 and 6. Using these values, the calculated values of ε at various wavelengths have been inserted in Figs. 2 and 3, as the full curves for values of (R/DNA) less than 0.4; tolerable agreement with experimental data is obtained. The dotted curves for values of (R/DNA) greater than 0.4 show that the values of β become progressively less than the possible maximum, and that the binding constant decreases as (R/DNA) increases. However, the effect of decreasing concentration of DNA is in this case (with no added Na^+ apart from that of the stock solution, *i.e. ca.* twice the concentration of DNA) to lower the concentration of Na^+ to a level at which "denaturation" of DNA can occur. This process is shown by the increase in extinction coefficient of the absorption spectrum of DNA[15], and results in a lowering of the affinity for binding of rosaniline[7].

The forms of the absorption spectra of bound "dimeric" and "polymeric" dye are qualitatively as expected. The occurrence of a subsidiary peak, towards the longer-wavelength end of the spectrum, derived for the "polymer", corresponds with that observed during the salting-out of similar dyes[16] and for crystalline hydrocarbons[17], and shown to occur as part of the metachromatic spectrum by MICHAELIS AND GRANICK[12]. In general, the present analysis may be said to confirm the view of MICHAELIS[1], that metachromasy results from the "dimerization" and "polymerization" of the basic dyes giving rise to absorption bands in the regions indicated.

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SUMMARY

1. The absorption spectra of rosaniline in aqueous solutions of sodium thymonucleate over the complete range of ratio of concentrations is reported.

2. The changes in spectrum of the dye are related to the proportions of dye bound according to a simple statistical analysis.

3. Absorption spectra for "dimeric" and "polymeric" bound rosaniline are deduced as an analysis of the metachromatic effect.

RÉSUMÉ

1. Les spectres d'absorption de la rosaniline dans des solutions aqueuses de thymonucléate de sodium dans tout le domaine du rapport des concentrations sont décrits.

2. Les modifications du spectre du colorant sont reliées aux proportions de colorant lié selon une analyse statistique simple.

3. Les spectres d'absorption de la rosaniline "dimérique" et "polymérique" liée sont déduits d'une analyse de l'effet métachromatique.

References p. 332.

ZUSAMMENFASSUNG

1. Das Absorptionsspektrum von Rosanilin in wässerigen Natriumthymonukleatlösungen im gesamten Bereich der Konzentrationsverhältnisse wird beschrieben.

2. Auf Grund einer einfachen statistischen Analyse werden die spektralen Veränderungen des Farbstoffes mit den Proportionen des gebundenen Farbstoffes in Verbindung gebracht.

3. Auf Grund der Analyse des metachromatischen Effektes werden Absorptionsspektra für "dimerisch" und "polymerisch" gebundenes Rosanilin gefolgert.

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